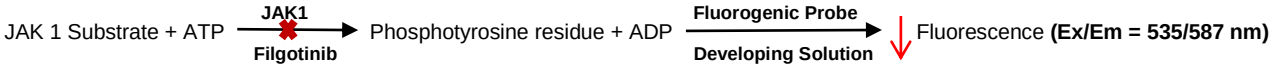
Janus Kinase 1 Inhibitor Screening Kit (Fluorometric)

11/21

(Catalog # K2111-100; 100 assays; Store at -80 °C)

I. Introduction:

Janus Kinase 1 (JAK1) is a member of the JAK family of intracellular, non-receptor tyrosine kinase that uses the JAK-STAT pathway in cytokine-mediated signaling. It has two mostly identical, phosphate-transferring domains where one domain exhibits the kinase activity, while the other domain regulates the kinase activity of the first. JAKs play a critical role in regulating diverse signaling pathways that govern cellular survival, proliferation, differentiation and apoptosis. Several JAK inhibitors including baricitinib (Olumiant), tofacitinib (Xeljanz), and upadacitinib (Rinvoq), are approved by the FDA to treat rheumatoid arthritis. Additionally, they are studied for the treatment of atopic dermatitis, essential thrombocythemia, polycythemia vera, psoriasis, alopecia, ulcerative colitis, etc. **BioVision's Janus Kinase 1 Inhibitor Screening Kit** is a 96-well plate based, fluorometric assay to screen potential JAK1 inhibitors. The kit uses an active, human JAK1 enzyme, which hydrolyzes the JAK1 substrate and ATP to generate ADP. ADP in the presence of a fluorogenic probe and developing solution yields a fluorescent product, which is measured at Ex/Em = 535/587 nm. However, when JAK1 activity is inhibited by a potent and selective JAK1 inhibitor, filgotinib, the fluorescent signal is reduced. The assay provides a rapid, simple, and reliable test for high-throughput screening of JAK1 inhibitors.



II. Application:

- Screening or characterizing Janus Kinase 1 inhibitors.

III. Kit Contents:

Components	K2111-100	Cap Code	Part Number
JAK1 Assay Buffer	25 ml	WM	K2111-100-1
Fluorogenic Probe	200 µl	Blue	K2111-100-2
ADP Detection Mix	1 vial	Purple	K2111-100-3
Developer Enzyme Mix	1 vial	Red	K2111-100-4
JAK1 Peptide Substrate	1 vial	White	K2111-100-5
Ultra-Pure ATP	1 vial	Orange	K2111-100-6
JAK1 Enzyme	1 vial	Green	K2111-100-7
Filgotinib	20 µl	Amber	K2111-100-8

IV. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- dH_2O
- Temperature-controlled plate reader

V. Storage Conditions and Reagent Preparation:

Store the kit at -80 °C, protected from light. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay.

- **JAK1 Assay Buffer:** Store at 4 °C or -20 °C. Warm to room temperature (RT) before use.
- **Fluorogenic Probe (in DMSO):** Divide into aliquots and store at -20 °C, protected from light. Prior to use, warm the solution to RT. After use, promptly retighten the cap to minimize the adsorption of airborne moisture.
- **ADP Detection Mix** and **Developer Enzyme Mix:** Reconstitute each vial with 220 µl JAK1 Assay Buffer. Divide into aliquots and store aliquots at -20 °C, protected from light. Avoid repeated freeze/thaw cycles.
- **JAK1 Peptide Substrate:** Reconstitute the vial with 220 µl ddH_2O to prepare a 50X stock JAK1 Peptide Substrate solution. Divide into aliquots and store at -20 °C, protected from light.
- **Ultra-Pure ATP:** Reconstitute the vial with 220 µl ddH_2O to obtain a 12.5 mM stock Ultra-Pure ATP solution. Divide into aliquots and store at -80 °C. Avoid repeated freeze/thaw cycles. Keep thawed aliquots on ice, while in use.
- **JAK1 Enzyme:** Provided as liquid. Divide into aliquots and store at -80 °C. Avoid repeated freeze/thaw cycles. Keep thawed aliquots on ice, while in use.
- **Filgotinib (3 mM):** Ready to use. Bring to RT before use.

VI. Janus Kinase 1 Inhibitor Screening Protocol:

Screening Compounds, Inhibitor Control and Background Control Preparations:

1. Sample Compound [S]: Dissolve the test inhibitors at 100X or higher concentration in an appropriate solvent. Further dilute to 10X using JAK1 Assay Buffer. Add 10 µl of diluted (10X) test inhibitors into wells of a 96-well clear plate with flat bottom labeled as Sample **[S].** Add 10 µl of JAK1 Assay Buffer into two wells labeled as Vehicle Control **[VC]** and Background Control **[BC]** respectively. For the **Inhibitor Control [IC]** well, prepare a 100 fold dilution of Filgotinib stock solution (such as mix 2 µl of Filgotinib stock solution with 198 µl JAK1 Assay Buffer) and add 10 µl of diluted Filgotinib into the designated well(s).

	[S]	[IC]	[VC]	[BC]
Test Inhibitor	10 µl	-	-	-
Diluted Filgotinib	-	10 µl	-	-
JAK1 Assay Buffer	-	-	10 µl	10 µl

Adjust the volume of all wells including Sample**,** Vehicle Control**,** Background Control and Inhibitor Control to **10 µl/well** using JAK1 Assay Buffer.

Notes:

a. Additional wells containing serial dilutions of the test inhibitors may be prepared, if desired. Each well should contain 10 µl of the test inhibitor at 10X the desired final concentration.

b. Organic solvents used to prepare the test inhibitor stock solutions may impact the JAK1 activity. To determine the effect of solvent on the JAK1 activity, we recommend preparing a parallel Solvent Control **[SC]** well with the same final concentration of the solvent used to solubilize the test inhibitor(s). If the signal obtained in the **[SC]** well is significantly different from the **[VC]** well, use the signal for the SC well instead of the VC well.

2. JAK1 Enzyme Solution Preparation: Prepare a 10-fold dilution of JAK1 Enzyme by adding 10 µl of JAK1 Enzyme to 90 µl of JAK1 Assay Buffer and mix well. Add 7 µl of diluted JAK1 Enzyme to each well containing Sample, Inhibitor Control, Solvent Control and Vehicle Control**.** Add 7 µl of JAK1 Assay Buffer to the Background Control well. Adjust the volume of all wells including Sample, Inhibitor Control, Solvent Control, Vehicle Control and Background Control to **60 µl/well** using JAK1 Assay Buffer. **Mix well and incubate at 37 °C for 15 min, protected from light. Note:** Discard any unused, diluted JAK1 Enzyme after use.

3. Reaction Mix Preparation: Prepare a 10-fold dilution of the Developer Enzyme Mix stock solution (i.e. dilute 2 µl of Developer Enzyme Mix stock solution with 18 µl JAK1 Assay Buffer and mix well). Mix enough reagents for the number of assays to be performed. For each well, prepare 40 µl Reaction Mix containing:

	Reaction Mix
JAK1 Peptide Substrate	2 µl
ADP Detection Mix	2 µl
Diluted Developer Enzyme Mix	2 µl
Ultra-Pure ATP	2 µl
Fluorogenic Probe	1 µl
JAK1 Assay Buffer	31 µl

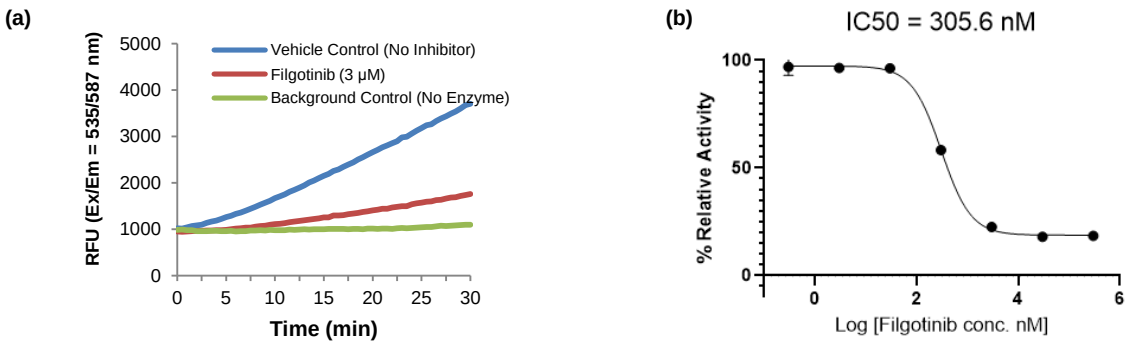
Mix well and add 40 µl Reaction Mix to Sample, Inhibitor Control, Vehicle Control**,** Solvent Control, and Background Control wells and mix well. ***The total reaction volume in each well will be 100 µl***.

4. Measurement: Measure the fluorescence immediately in kinetic mode for 30 min at Ex/Em = 535/587 nm at 37 °C. Choose any two time points (t_1 and t_2) in the linear range of the plot and obtain the corresponding RFU values (RFU_1 and RFU_2).

5. Calculation: Calculate the slope for Sample [S], Vehicle Control [VC], Solvent Control [SC] and Background Control [BC] by dividing the ΔRFU (RFU_2 - RFU_1) values over reaction time Δt (t_2 - t_1). Subtract the slope of the Background Control [BC] values from the [S], [VC] and [SC] values. *If the [SC] slope is significantly different from the [VC] value, use the [SC] value instead to calculate the effect of the test inhibitor(s).*

$$\% \text{ Relative Inhibition} = \frac{\text{Slope of [VC]} - \text{Slope of [S]}}{\text{Slope of [VC]}} \times 100$$

$$\% \text{ Relative Activity} = \frac{\text{Slope of [S]}}{\text{Slope of [VC]}} \times 100$$



Figures: (a) Reaction kinetics of recombinant JAK1 enzyme at 37 °C in the presence and absence of filgotinib. (b) Inhibition of JAK1 activity by filgotinib. IC_{50} of filgotinib was calculated to be 305.6 ± 0.9 nM. Assay was performed following the kit protocol.

VII. Related Products:

Janus Kinase 1, Human Recombinant (P1678)
EZSolution™ Filgotinib (B2481)
c-Src Kinase Inhibitor Screening Kit (K2015)
Filgotinib (2871)
DiscoveryPak™ JAK Inhibitors Set (S236)
STAT1 (His-tagged), human recombinant (7889)

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